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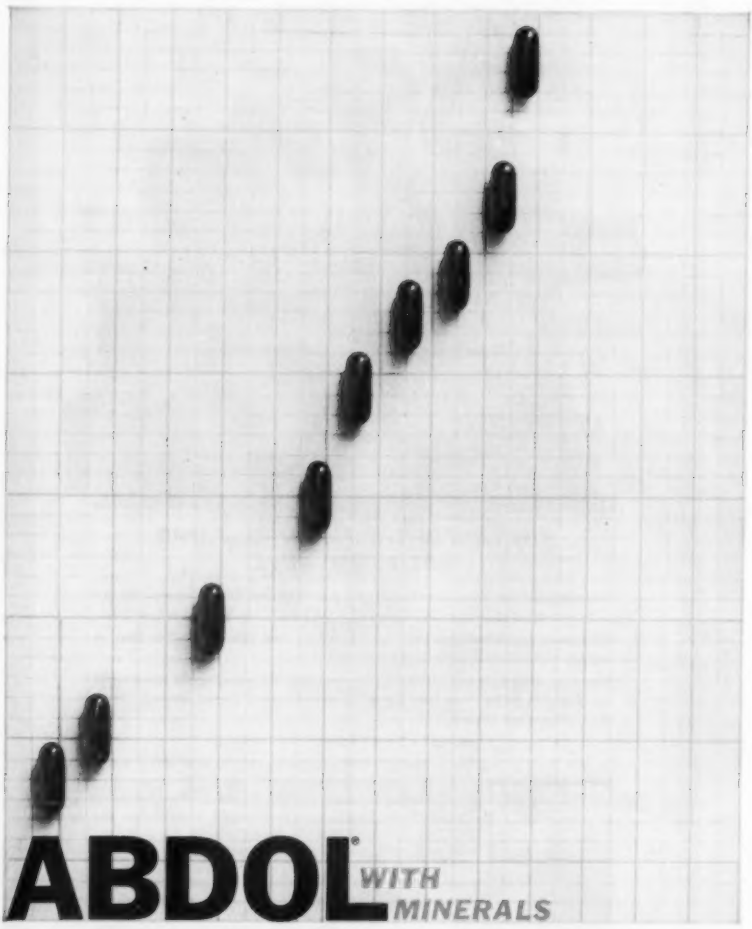

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E D I T O R I A L

THE NEED FOR PROFESSIONAL ORIENTATION IN PHARMACY

OVER the years, we have heard continued exhortations urging pharmacists to be better businessmen and to keep abreast of developments in distributive patterns if they hoped to survive. While not all pharmacists did all that might be done in this direction, few have failed to accept these admonitions at their face value and still fewer would deny their importance. There is no question that retail pharmacy has been fighting and will continue to fight for its survival in the face of mounting competition from supermarkets, department stores, discount houses, door-to-door selling, and all of the other developments in this rapidly changing field. Pharmacists, however, have been so preoccupied with this competition and so engrossed in their efforts to meet it that many have failed to recognize that their ultimate source of strength, security, and salvation lies not in being better businessmen—important though this may be. There are many signs on the horizon which point to troubled times insofar as the business of pharmacy is concerned and here we need only to consider the very serious and present threat to Fair Trade. There are those not gifted with optimism who see this cause already lost and, should it be, the cutthroat competition which would ensue will make a shambles of many small business establishments.

In looking objectively at what indeed is pharmacy's most critical problem today, it is not, however, the possible loss of Fair Trade. Important though this is, there are other signs and symptoms of greater significance. The attempt, for example, by certain agencies of government to establish the principle that pharmacy is only a trade and not a profession is far more serious. One wonders whether indeed it has not been the pharmacist's own efforts to be a better businessman which have led to this misinterpretation by those outside the realm of pharmacy.

We wish to suggest with all the emphasis at our command that pharmacy must immediately become more professionally oriented and

that its practitioners must think first in these terms even though at times it may result in some sacrifice of their commercial instincts. The day may not be far off when the only thing which we will have left is that professionalism that we have managed to preserve. Indeed, the day may soon be upon us when the profession of pharmacy will be the only thing upon which we may build our future. Pharmacy is undergoing changes which some might describe as "evolutionary" and others by the more drastic term "revolutionary." If we as a profession and as individuals are to be fully prepared for these changes as well as prepared to maintain the identity of pharmacy, many now in practice will find it necessary to reorient their thinking by placing the profession of pharmacy uppermost in their minds and plans—radical though this change may be.

L. F. Tice

EVALUATION OF ANALGESIC SUBSTANCES *

By Eaden F. Keith

Hoffman LaRoche, Inc., Nutley, New Jersey

THERE has been much discussion in the past about pain, and about laboratory methods of its production and evaluation. In this report, we will consider the laboratory evaluation of analgesic agents. Present-day evaluation of this activity is dependent upon the insight gained and the information gleaned from laboratory evaluation of the concepts proposed.

The primary objective of analgesia is the alleviation of pain, or suppression of the perception of pain in the human being. Here, we are not primarily interested in the latter aspect of the problem. We are interested in methods which evaluate potential analgesic agents from a laboratory point of view. If we define pain in this context as the perception of a noxious stimulus, which calls for an escape reaction, we can proceed.

In the being capable of having an abstract view, pain may be defined as the esthetic experience associated with the perception of a noxious stimulus. Since the perceptual component can be evaluated only in man, we must limit ourselves to a consideration of the reaction patterns to noxious stimuli in laboratory animals.

If we observe these animals closely, we obtain experience in evaluating these reactions and results may then be useful in the various techniques that have been suggested. To best utilize these techniques, we must first establish a series of criteria for producing and measuring reactions, and the evaluation of analgesics will then be based on the criteria that we establish. Therefore, we will propose four criteria that might be used in experimental procedures.

First, we should establish as a limiting factor that the measurable aspect be related to the pain sensation.

A second criterion is the requirement that the stimulus produce a response which is measurable and relatively constant. By being relatively constant, it is meant that the threshold of response should be within limits which are a fraction of the stimulus duration required

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to produce serious damage to the animal. This does not mean that the stimulus cannot produce damage, since it has been shown that the least stimulus that can be interpreted as pain will, if persisted in, produce damage in a particular area. Thus, any painful stimulus that we select will produce some minimal damage in order to elicit a response to "pain".

It follows then that the third criterion would be that the stimulus used should be a minimally damaging fraction of a potentially injurious stimulus.

Fourth, the stimulus should be easily applied and readily controlled, and, ideally—which has not been attained in any of the techniques which have been proposed up to the present time—the stimulus should produce a single sensation; that is, the perception of a pricking, burning, or aching type of pain. None of the stimuli that are known to have been utilized for eliciting a response do precisely that. There is always an ancillary sensation which will be perceived by the subject, in association with the painful stimulus.

Several theories have been proposed concerning the nature of pain; two have survived. The first to be cited is the intensive theory. This theory is based on the hypothesis that all sensory endings give rise to pain if the stimulation or the stimulating quality is of sufficient intensity to produce a maximal or a just submaximal response of this ending.

There are several investigative procedures which have shown that the intensity or frequency of response of a particular receptor can be attained without the production of pain. A classical experiment in this regard is one in which blasts of air have been directed on the skin of a frog and the spike potentials in the nerve trunk leading from the area have been measured. Maximal production of these spikes has been recorded and the animal has shown no tendency to react as one would expect to a painful stimulus; yet, this same animal, when stimulated in the same area which has been exposed to the strong blast of air, responds immediately to the prick of a pin or to pinch, so that the painful stimulus is very definitely divorced from the sensation of air blowing upon the area.

The sensation theory, which states that pain is a sensation having its own central and peripheral mechanism, has a great deal of scientific evidence to support it, both from the standpoint of physiological and anatomical investigations. Clinical investigations have also given rise

to the same conclusion. Areas which have been studied histologically, and have failed to give pain reaction, have been sensitive to heat, have been sensitive to touch, and the endings to which the perception of these particular moieties of sensation have been attributed are present. The free nerve endings which have been associated with the reception of pain are absent.

Anatomically, the pain receptors are the arborization of axons having free endings terminating in the dermis and, according to Tower (1), a single axon supplies an area of about one centimeter in diameter.

There are two modalities of pain which are derived from superficial areas and these are as follows: The sensation of pricking pain, electrometrically, reaches a sharp peak and subsides quickly. The second described as burning pain has a slow onset, it rises gradually, it tends to plateau, and it never reaches the same type of peak nor intensity that one observes with pricking pain. Burning pain also persists for a longer period of time and recedes slowly.

In humans, the pain threshold has been delineated and is about two thousand times the threshold determined for heat perception. At the level just producing pain, there is a distinction made between heat and pain, and there is a very definite line of demarcation which can be detected by the individual able to let us know exactly what he is feeling.

The question that always arises in the mind of the investigator working with animals is whether or not the animal is reacting to a threshold of heat or to a painful stimulus. Later in the course of this discussion, we will consider this point, but there are indications that there is a distinction being made.

In the selection of techniques which are to be used for the identification of an analgesic agent, we must decide on the type of analgesia that we wish to have. Most of the techniques that we have for eliciting reactions are designed for a specific type of painful sensation. This has been appreciated for a long time. In 1948, Pfeiffer *et al.* (2) classified pain in the following three categories.

"Supain" is the term used for the description of superficial pain and this pain is of a nature elicited most readily by the technique originally described many years ago by Von Frey. It is the type of pain referred to previously as pricking pain. One can also elicit it by applying a needle point with sufficient force to the skin.

The second category is *"Deepain"*. This is pain of an aching character. It is described as the pain associated with a toothache. It is the type of pain in which "protopathic nerve fibres" are said to carry the impulse.

A third category of pain—and it should be mentioned at this point that we have no really good method for evaluating agents which affect this type of pain—is *"Sympain"*—the pain of structures such as the vessels of the temporal region superficially, and of the vessels supplying the brain as an example of deeper structures. Pain of this type is seen in migraine headaches, and is frequently relieved by ganglionic blockade.

The levels at which we can appreciate pain blockade, going from the periphery to the central nervous system, are the receptors themselves; agents which will block pain or will produce analgesia, when acting superficially, will act at the level of the receptor itself. A second area where analgesia may be produced is in the internuncial pool of the cord either by blockade or raising the threshold for synaptic transmission. Proceeding up the cord, the thalamic area may be involved in the suppression of painful stimuli; or, at, a fourth level, the cortical appreciation of pain may be obtunded. In pain, all these areas might definitively be altered in order to obtain analgesia. Vascular pain may be obtunded at the receptors, at the ganglia, or in the central nervous system proper; i.e., in the cord, at the thalamic, or cortical levels.

We are aware of the fact that muscle and joint pain can be obtunded by a certain group of analgesic agents. The "blockade" (?) by this group of substances—reference is made here to derivatives of salicylic acid and the coal tar series which relieve pain from deep structures in muscular or joint areas—is probably produced at the receptor. There may also be a factor in the central nervous system at the various levels previously mentioned.

If we are to select an analgesimetric technique, we must consider whether we want an agent which attacks pain at the central nervous system; that is, in the cord, in the thalamic area or at the cortical level, or one acting peripherally. In the remainder of this presentation, we will refer to potent analgesics as the class that act in the cortical area and will refer to those of the salicylic acid derivation and compounds of similar potency as weak analgesic agents which act at the periphery, at least in so far as we have been able to determine from a laboratory point of view.

A group of materials, which may be referred to as the broad-spectrum analgesics, occupy a "No-Man's Land." There has been very little done in the way of developing a method for specifically determining activity for compounds of this range of potency. This refers to compounds which are at the level of analgesic action of codeine and d-propoxyphene or Darvon ®. We must decide which of these areas we want to work in at the time that we are selecting an analgesimetric technique.

We must then decide whether or not the end point in the animal is one that is intimately connected with the pain syndrome. In other words, is this reaction related to an ancillary stimulus response in the symptom [1] complex elicited by the presence of the painful stimulus, or is it one that is related closely to the symptom complex for *pain?* The closer we can get to the symptom complex for pain, the closer we are going to be to selecting a compound which will be potent in the clinical application of this compound.

If the technique is simple, we are going to be able to study a large number of compounds. This is one of the major problems in industrial pharmacology, at least in the large operation. If the technique is involved, we are not going to be able to cover a large number of candidate compounds. We must select our technique for the observation that we wish to make in accordance with the load that is going to be placed upon us in the program design. Once we have decided on these things, then we have a broad background of techniques from which to select. Some of them are good; some of them are not so good. Some of them are primary screening techniques; some of them are definitive. The selection of primary techniques and selection of definitive techniques will depend largely upon the individual who is overseeing the analgesic program and the decisions he makes in regard to the previous considerations cited.

Testing Techniques

A. *Chemical Methods*

There have been five modes by which pain responses have been elicited. The first one that we will consider is the chemical technique of eliciting pain.

1. The term is not accurately used since "signs" are really what we see in animals.

1. A very simple method is a frog foot immersion technique in which the skin of the frog is exposed to an acidic solution producing the response of withdrawal in a period of about five seconds. The pH of the acid solution is adjusted to elicit a response in approximately this period of time. An agent is administered and, if it is an analgesic agent, we will see a prolongation of immersion. The comparison is one of a temporal type in which the length of immersion is proportionate to the degree of analgesia produced. Alternate foot immersion will serve to maintain a minimal degree of local damage.

Another technique which has been used has been the intraperitoneal injection of acid in mice. This produces a writhing response and the inhibition of the writhing response is used as an end point.

2. Croton oil is an extremely irritant material when applied to the skin. It has been used as a painful stimulus in primary screening for the assay of analgesic activity. The introduction of a small volume of croton oil in the subcutaneous area produces a rather intense response, and the animal will attempt to bite, will show increased activity, and the inflammatory responses will appear at the site—that is, the response of rubor, tumor, calor, and dolor will appear and can be evaluated in this approach. This is a multiphasic type of evaluation and considers not only the analgesic component, but also the other components which are characteristic of antipyretic analgesic substances.

3. Until recently, the chemical methods for analgesimetry or for producing pain have been largely overlooked or held in ill repute since they are difficult to evaluate and they are ordinarily subjective tests. Recently, phenylquinone has been introduced as a writhing agent and work has been done with phenylquinone in several laboratories to determine whether or not it is a good chemical method for evaluating analgesic substances. Siegmund, Cadmus, and Lu of the Ethicon Laboratories first described this test (3). In this technique, a 0.02 per cent concentration of phenylquinone in 5.0 per cent aqueous solution of ethyl alcohol is made. Introduction of 0.25 ml. of this material into the peritoneal cavity of the mouse produces a characteristic writhing response. This writhing response occurs five or more times in a period of ten minutes immediately after administration. If the animal does not show the response five times in a period of ten minutes, the animal is not used in the evaluation of drugs.

Mice which are not treated with an analgesic substance will show this writhing activity, which consists largely of dragging the abdomen on the cage, a tendency to work to one or the other side of the cage with the hind feet raised or a hind foot drawn in against the side of the body—a rather characteristic reaction which, once seen, is readily recognized.

The animals that are exposed to graded doses of a new substance are observed for five-minute periods at fifteen-minute intervals. Those that show the writhing response in this period of time are removed. At the end of the experimental time set, the ED_{50} of the material can be estimated by counting the number of animals at each dose level which have failed to show the writhing response. These animals which have not shown the writhing response are considered to have experienced analgesic effects.

Compounds that were tested in our laboratories and were also reported in the paper by Siegmund and co-authors (3) have been compared (Fig. 1). However, a larger number of animals than reported by these authors have been included in the group from Hoffmann-La Roche Laboratories.

In our laboratories, morphine has an ED_{50} of 1.5 mg./kg., which is essentially the same value obtained in the Ethicon group's series and d-propoxyphene has an ED_{50} of 15 mg./kg. Acetylsalicylic acid in our series has an ED_{50} of 38 mg./kg.; whereas, Siegmund's results show an ED_{50} of 165 mg./kg. for the same compound. The quantitative discrepancy here is not readily explained. However, the

PHENYLQUINONE WRITHING TEST

	H. LaRoche	*Ethicon*
Morphine	1.5 mg./kg.	1.15
d-Propoxyphene	15	——
Acetylsalicylic Acid	38	165
Sodium Salicylate	250	——
Aminopyrine	150	98
Butazoladine	400	——
Xylopropamine	16	——
Tremorine	4.5	——

Figures Express ED_{50} for Substance Listed

FIG. 1.

basic point to be made is that the activity can be detected. This certainly demonstrates that these weaker analgesic substances can be picked up in this test at a lower dose than has been possible in the techniques that have been previously described.

Sodium salicylate, which is probably the weakest analgesic agent as far as laboratory investigations are concerned, can be detected, but it has a high ED_{50} of 250 mg./kg. in this test.

Another divergence of results appears. The ED_{50} for aminopyrine was found to be 98 mg./kg. by the Ethicon group and 150 mg./kg. in our laboratory. Whether or not these findings are due to broader spread of dose or a smaller number of animals can not be stated but, since statistical evaluation of the results are not available, it is difficult to attempt an explanation of this difference.

Xylopropamine is a chemical compound which, when given to animals, produces analgesic effects. In the laboratory examinations that it has been subjected to so far, it shows an ED_{50} comparable to that of d-propoxyphene. Although the compound was synthesized some four or five years ago, it has not at this time been adequately evaluated in clinical studies. Studies have only been continued because of its interesting activity.

Tremorine was used in one of the tests for the production of analgesia. It was included in the group of compounds screened and had an ED_{50} of 4.5 mg./kg. The response of the animals would be interpreted as analgesic activity. It would seem that this would demonstrate a lack of specificity for the writhing technique as an assay.

The mechanism for the production of writhing in the mouse has been studied by Eckhard *et al.* (4). They concluded that histamine, or the release of histamine, was not the mechanism responsible for the writhing response in the rat and probably not responsible for the response in the mouse.

The question has been raised as to whether or not the acidity of the solution of phenylquinone produced the response. Eckhard *et al.* (4) showed that pH was not responsible for the reaction. This group proposed that it may be the release of some substance other than histamine, serotonin, heparin, or bradykinin which instigates the writhing response. They leave open the possibility that direct irritation by the substance itself is the mechanism. The conclusions derived from the studies by Eckhard and his group were that

phenylquinone and hydrochloric acid were the best agents for the production of writhing, if this test is to be used as an analgesic screening procedure.

Opiates and nonopiates can be evaluated using this technique and of the two agents, phenylquinone and hydrochloric acid, phenylquinone is the better since the writhing syndrome produced by the latter material has a longer duration of action than does hydrochloric acid.

B. *Electrical Methods*

1. The second category of tests which can be utilized for the evaluation of analgesic substances are those which use electrical methods of producing noxious stimulation. This method of painful stimulation of skin was originally described in 1851 by von Helmholtz. Variations of the technique have been applied over a period of sixty or seventy years for evaluation of analgesic action. The guinea pig is frequently used; however, the threshold of stimulation required for eliciting a painful response in this animal is too high. Secondly, the nature of the guinea pig is such that the response elicited—a squeal—can be produced in this animal by handling alone. The third objection is that a sensation which precedes the actual painful stimulus itself could elicit the response. Obviously, the end point here is one that is not sufficiently specific.

2. Macht and Macht (5) describe another technique involving the application of an electrical current to the rat scrotum. The adult male rat has been found to be extremely sensitive to this type of stimulation. Fine platinum electrodes are applied to two marked points on the scrotum of the rat and the current is increased until the animal squeals. They describe six points which can be utilized for application of the current: over the body of the testes, over the tips of the testes, or over the proximal or distal end of the septum.

They state that superficial pain can be distinguished from deep pain by eliciting a squeal which develops with current of sufficient intensity to produce the end point without producing a cremasteric reflex. The cremaster muscle is a muscle sheath which upon contraction normally squeezes the testes into the abdominal cavity. Its contraction can be powerful. If deep pain is to be elicited, current must be applied which is strong enough to evoke a contraction of the

cremaster muscle. This contraction in turn produces pain by exerting pressure on the testes within the scrotal sac.

By watching the response of the animal and knowing that pain is prevented at levels which ordinarily would produce a squeal before the cremasteric reflex occurs, they know that they have obtunded superficial pain. If at the time of cremasteric response the squeal occurs, they know that superficial pain has been obtunded but deep pain is still present. If the cremasteric reflex is elicited and the animal does not squeal, they know that they have suppressed both the superficial and deep pain. In this way, Macht and Macht concluded that they could distinguish between the weak and potent analgesic substances. Whether or not this is a valid conclusion is still open to question.

3. Another method was developed by Reinhart and De Beer at Wellcome Laboratories. They were not satisfied with the reproducibility of results. Consequently, they have not published it formally. However, J. H. Burn of Oxford thought sufficiently well of the technique to report it in his text, "Biological Standardization" (6). In this method of evaluation, two electrodes are placed on the tail of the mouse which has been prepared by application of electrode jelly. Care is taken to be certain that surface conduction is prevented between the two electrodes. The distance between the electrodes should be 1.5 cm. The stimulating system consists of a contact wheel which revolves once a second and delivers a shock of 1/26 of a second duration at 6 volts. Control animals in this situation will squeal between the third to fifth shock. If at five shocks the animal does not squeal, this animal is eliminated from the evaluation procedure.

With this technique, Reinhart and De Beer have been able to show a linear relationship between log dose and what they describe as a corrected score. The corrected score is calculated by multiplying the least response or the least time elapsing between the beginning of shocks and the first response in the test group—that is, the least number of shocks delivered (L) times the mean of the test group (M_1) minus the mean of the control group (M_2) divided by the mean of the test group. $L \frac{M_1-M_2}{M_1} = C.\ S.$ For the standard

analgesics that have been tested with this technique, and by applying the corrected score, a linear log dose relationship can be shown.

The technique is sufficiently sensitive so that analgesics with activity in the order of phenazone and papaverine have been detected by this technique in Burn's laboratory.

4. Another electrical technique which has received widespread use and which is remarkably sensitive is the tooth pulp method. It was first described by Koll and Reffert in 1938 (7). A year later, Ruckstuhl (8), in Bern, did some definitive work on the technique, outlined difficulties encountered, and eliminated some difficulties that originally were cited by Koll and Reffert. Fleish and Dolivo, in 1953 (9), further modified the method. Their techniques were further improved by Yim *et al.* (10). With the modifications that have developed over the years, some of which Harris (11) has contributed, the method is used rather extensively at the present time. The technique consists of delivering to an inlay or filling in a vital tooth a shock at various levels of intensity in animals or in human beings. The threshold of pain is determined. The alterations in threshold of pain produced by various substances, weak analgesic agents among them, can then be determined in the animal and in the human.

Because of the application to the human and the conditioning that humans and animals may undergo in a test situation, Harris has rigged an ingenious device to confound the subject. Noiseless buttons are hidden from view under a table and a contact switch is constantly closed. The actual shock is delivered only when the hidden button is depressed and it is usually depressed in synchrony with a constantly operating audible contact. In this way, he is able to ascertain within very narrow limits, in both the animal and in man, the thresholds for pain under various circumstances.

One of the limiting parameters of evaluation with the tooth pulp method is the proximity of the filling to the pain receptors. In animals such as the rabbit, when the filling is placed in the incisor teeth, one must replace the fillings approximately every two weeks because of the rapid growth of the incisors. As a result of growth, the filling is pushed away from the pain endings—and, from experiment to experiment, if the series is conducted over a long period of time, the threshold alters. Consequently, the threshold has to be continually re-established.

If the molars are used, and they do not grow quite as rapidly as the incisors in the rabbit, an error can be introduced by saliva on the tooth. Thus, the tooth must be dried, and kept dry, during the period of experimentation. This is also true if the human is the experimental subject. The dryness of the area surrounding the tooth prepared for experimentation is something that must be carefully maintained.

The response of the rabbit in this method of evaluation is a chewing response which begins immediately upon perception of the stimulus. In the dog, it is not quite so definitive a reaction. The dog may bark, or there may be a contraction of the muscles in the facial region, or the chewing motion may also occur. Any or all of these responses may occur in a single animal. Therefore, the subject must be watched carefully.

The attachment of electrodes requires care since good contact is imperative. The method for making contact is usually a clip type of applicator through which the wire is connected to the filling. One method of preparing a clip is to take an ordinary safety pin, cut off the head, and bend each arm at right angles in three places so that the spring portion of the pin is below the crossed arms and the two points meet when the pin is in the resting position. With this clip, the contact can be made with certainty each time that it is applied. An indifferent electrode is applied to the body surface with electrode paste to assure contact.

Any experimental approach to the study of analgesics must take into consideration the problem of conditioning the animal to the stimulus. Having this in mind makes one aware of the necessity of randomizing the application of the stimulus.

C. *Mechanical Methods*

The mechanical techniques for producing pain are the next group which we will consider. As a group, these techniques are best applied, in all probability, to primary screening methods. Since the evaluation of results obtained with these methods is largely subjective and, since they are not readily quantitated, the methods should be restricted to primary screening.

1. One of the techniques that is used requires a Rochester-Pean straight surgical forcep having a long blade with some spring to it.

The blades should be covered by tygon tubing to prevent the serration on the blades from actually damaging the tissues of the animal. An area is painted on the tail of the animal for application of the forceps and an area marked off on the forceps so that the same area on the tail and the same area on the forceps are used each time. The amount of pressure necessary to elicit pain is expressed in the number of clicks on the box lock which are required in order to produce a squeak in the mouse. This value can be used as an indication of the degree of analgesia or the level at which the painful response is elicited in the animal.

2. The use of toothed forceps has also been employed in which a dynamometer is interposed between either the handles or the blades in order to determine the amount of the pressure that is required to produce a response, utilizing the same area of the skin or the same area of the tail of the animal to elicit the reaction each time.

3. A recent method described for determining analgesic activity is the application of an artery clamp, the so-called "bulldog clamp", to the tail of the mouse and measuring the length of time it takes for a mouse to attempt to remove the clip from the tail, this latent period of attack on the "bulldog" clip indicating the degree of analgesia produced in the animal. For general screening techniques, these may be satisfactory.

4. In the human, the mechanical techniques are many, using areas over bone for application of pressure, the use of the Von Frey hair, a technique in which the diameter and length of the hair is measured and the amount of the pressure required to bend the hair is calibrated. A sharp point is fixed to the end of the hair and it is applied to the skin. These hairs are applied serially until pain is elicited without flexion of the hair. This establishes the threshold for pain. Hairs similarly calibrated having greater flexion resistance will establish the level for perception of pain after administration of an analgesic. Modifications made by Seevers *et al.* (12) and some that others have introduced make the test more precise than in the original description.

There are techniques for eliciting visceral pain. Pressure is produced in the enteric canal (or in the esophagus) by introducing and inflating balloons. A manometer is interposed to measure the pressure

in the system. A feeling of pressure, or substernal pain in the case of the esophageal balloon, is reported by the subject. However, these techniques are not particularly good for evaluating pain in the animal since the animal cannot tell you when he feels pressure or pain. We might be able to elicit a response in animals in this way but the end point would be difficult to establish. These latter mechanical techniques are mentioned only briefly since they are not applicable to the laboratory screening of analgesic compounds.

D. *Thermal Methods*

1. Thermal techniques make up the fourth category. Of these techniques, the one which is the simplest is the hot plate technique originally described by Woolfe and MacDonald and later modified by Eddy. Woolfe and MacDonald (13) used a zinc plate with a lamp beneath it. Eddy modified it to use an electric lamp as a source of heating and a copper plate for the conduction of heat. Later, Eddy (14) came to use a mixture of equal parts of ethyl formate and acetone. This mixture boils at a temperature of 55 ± 0.5° C. Eddy used 1200 cc. of fluid in his apparatus and the material lasts for several months since the evaporate is refluxed by means of a condenser column about 3 inches in diameter and 12 inches in length. The solution maintains the desired temperature range throughout a period of several months. A beaker with the bottom cut out is placed on the surface of the plate so that the animal cannot escape.

The first sign of discomfort with these animals is usually an attempt to sit up and lick the forepaws. This is taken to be indication of the pain threshold. The indication of unbearable pain is said to occur when, in the undrugged state, the animal begins to dance and jump about. Drugged animals, in this situation, instead of jumping about, will frequently withdraw the hind paw and hold it close against the abdominal wall and alternate paws in this position.

The average reaction time for 2000 untreated mice was 9.51 ± 1.02 seconds, which indicates that 99+ per cent of normal mice would react within 13 seconds. Thirty seconds is the "cut-off" time for drugged mice so that injury to the feet of the animal will not occur.

This technique is widely used. It is a good preliminary screening technique and there have been claims made that this technique will

detect the analgesic activity of weak compounds when administered at high dose levels.

2. The hot wire technique of Davies, Raventos, and Walpole (15) was described in 1946. This consists of the application of heat from a coil of wire recessed in an asbestos plate in which a channel has been made for placement of the animal's tail. Current is applied to the wire and the time from the start of current flow to the withdrawal of the tail is taken as reaction time. The heat must be applied not more than 1½ inches from the tip of the tail and the rate of heating of the wire is adjusted so that the animals react in 5 seconds.

The technique described gives the dose response curves, shown in Fig. 2, for morphine, codeine and pethidine (meperidine), administered by the intravenous route. From this graph, it is obvious that this is an effective method for the evaluation of the potent analgesic substances.

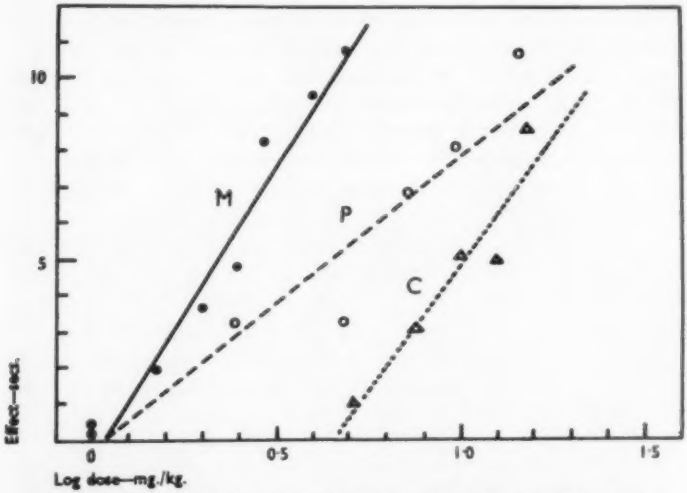

FIG. 2.—Analgesic action of morphine (M), codeine (C), and pethidine (P) administered intravenously. Effect at 15 minutes after injection plotted against log. dose.

FIG. 2.*

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3. The method of D'Amour and Smith (16), which was described originally in 1941, is one in which the light from an automobile headlamp is focused on the tip of the tail of an animal to produce heat, and the time measured between the application of the heat and the flick of the tail. This is "reaction time." The stimulus is adjusted so that controls respond within a 5-second period, and the dose of a compound that prevents response for a 10-second period in a fraction of the particular group of rats is reported as per cent analgesia. In other words, if one has 10 animals to which a dose of a compound is given, and only 4 of the animals respond within the 10 seconds, it then is considered that 60 per cent of these animals experienced analgesia and the other 40 per cent did not.

In 1943, the technique was modified by using a scoring system. The animals that responded, in 7 to 7.5 seconds, were given a score of +1; from 8 to 8.5 seconds, of +3; from 9 to 9.5 seconds, of +6; and no response in 10 seconds, +10. The degree of analgesia is estimated as the sum of the scores of all animals receiving a specified dose. With this method, it is possible to detect analgesic effects with large doses of aspirin and aminopyrine. However, large doses are required to show even a slight degree of analgesia. Burn considered that this method was satisfactory for the detection of the potent analgesics, but that it was unsatisfactory as a method for determining the activity of the weaker analgesic agents.

A further modification of this technique was proposed by Thorp (17). He used a modification of the D'Amour-Smith method in which he exposed the animals in a conditioning experiment to the effects of heat focused on the tip of the tail. Application of the high intensity stimulus several times conditioned the animals to respond when they felt a painful stimulus. When the stimulus was repeated at a lower level, the animals were watched to see whether they failed to respond. Thorp believed that he was training the animals to distinguish between the heat stimulus and pain stimulus. With the trained animals (those responding to high intensity heat, but not to low intensity), he proceeded to evaluate a substance for analgesic activity.

4. The Ercoli-Lewis test (18) is a modification of the same basic principle. The apparatus consists of a 1000 watt bulb focused on the loin of the animal. The animal is protected by a plexiglas shield in which a port has been cut, and a shutter placed between the lens and

the animal. When the shutter is opened the timer starts. When the animal reacts, the shutter is closed and the timer stops. The reaction time is thus determined.

Ercoli observed that during the exposure period there were two reactions. One was the reaction of skin twitch in which the animal's skin began to "creep", due to the action of the cutaneous maximus muscle; the other was a "flight reaction", which he described in the paper on this method.

The strength of the incident light is controlled by adjusting the voltage of the lamp circuit with a rheostat so that the heat delivered per unit time could easily be controlled. This further modification of the thermal technique permitted Ercoli to develop several analgesic agents. It is an excellent method for the detection of potent analgesic substances.

5. Later modifications of this test were reported by Hardy, Stoll, Cunningham, Benson, and Green (19). They developed a technique which takes into consideration not only the incident light but also the physical properties of the skin and the temperature changes in the skin of the animal. These factors are considered in the estimation of the activity of analgesic agents when their modification is used.

Figure 3 depicts the apparatus, the elements of which are made by various companies, and which we now use in the evaluation of

FIG. 3.

potent analgesics. The gun at the right front is the source of light. The potentiometer is used for calibrating incident light (heat delivered), and an automatic timer is interposed in the system. The Stoll-Hardy dolorimeter is used to control the current delivered, thus controlling incident light. The incident light applied to the rump of the animal yields 329 millicalories/sq. cm./second in our test procedures. This stimulus is so adjusted that it permits assessment of the strength of various compounds which show analgesic activity. The Leslie cube is used for calibrating the thermocouple. The thermocouple is kept in a cold body and used for determining the temperature of the animal's back before the application of the stimulus. By application of the formula, we are able to calculate the temperature of the animal's skin at the time of the withdrawal response. We use the withdrawal response rather than the skin twitch because we feel that it provides an index of pain rather than skin reflex response.

The results of some experiments that have been completed using this instrument are presented in Fig. 4. In this, we see the reaction times for several substances: Morphine, Levorphan, and Azepetine (Ilidar ®). The latter is an adrenergic blocking agent used because of its ability to lower skin temperature. The drop in skin temperature was associated with an increase in the reaction time but, when the corrections were applied, the temperature required to produce the withdrawal response in control animals was exceeded, but not significantly above those of the control.

Aspirin was used in order to determine whether or not the test was sensitive enough to detect the analgesic activity of aspirin. At 1000 mg./kg., no effect could be observed.

The increase in local skin temperature at withdrawal following the application of the heat stimulus is shown in Fig. 5. These are calculated temperatures. The temperature of the skin is taken before the heat is applied and, by use of the factors previously determined, the skin temperature in the area of stimulation at the time of withdrawal is calculated.

At the time of withdrawal following morphine, the temperature of the skin was approximately 62° C. The normal range of skin temperature at painful response—that is withdrawal—is generally between 51° and 54° C. This demonstrates that, in an animal medicated with a potent analgesic substance, the temperature of the skin at the withdrawal response is markedly raised. Levorphan tartrate

at 5.0 mg./kg. subcutaneously raised the skin temperature at the withdrawal response to approximately 60°. Thus, by using this technique, it is possible to evaluate the analgesic activity in terms of the temperature required to cause withdrawal. The per cent increase in time is shown in Fig. 4. In the case of materials lowering skin temperature, the temperature at withdrawal remains in the normal range. Therefore, the response temperature is a more critical index of the pain threshold than is the reaction time. Reaction time is lengthened, of course, because additional time is required to bring the temperature of the skin to the threshold temperature at a constant rate of heat application when the skin temperature after drugging is below non-medicated skin temperature.

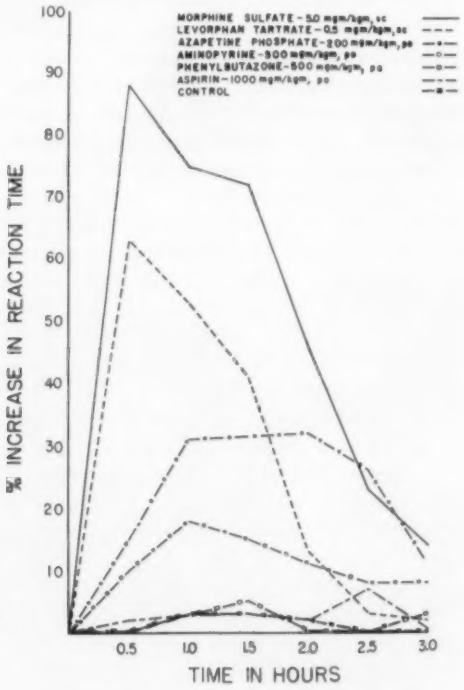

FIG. 4.*

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In this, we have a technique which is useful in the evaluation of the potent analgesic substances. It is probably more critical than any of the techniques that have been proposed heretofore.

E. *Pharmacological Methods*

1. The next group are the pharmacologic tests for antipyretic analgesics. The antipyretic tests are those in which a substance is administered to produce a rise in temperature of the animal. The skin temperature may be used, or the core temperature; i.e., rectal temperature of the animal, may be used as the index. Yeast is a good substance for producing an increase in temperature, or proteose-peptone mixtures will usually result in an increase in body temperature. The ability of a compound to inhibit this induced rise in temperature has been used as a means for evaluating antipyretic analgesic activity.

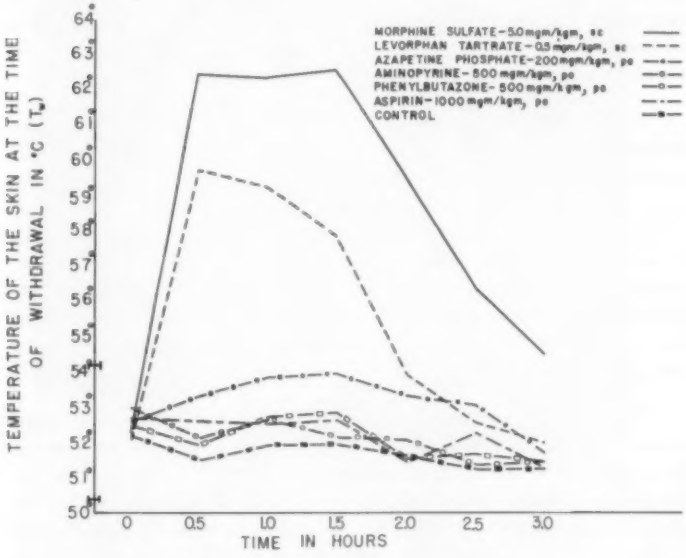

FIG. 5.*

* Reprinted, with permission, from *J. Appl. Physiol., 11,* 459 (1957).

2. Another technique is the potentiation of codeine analgesia, the administration of a combination of codeine and test substance in order to detect synergistic or additive action. This may aid in assessing analgesic activity of the substance combined with codeine.

3. A general observation which can assist in selecting compounds having analgesic activity is the Straub tail effect. Spinal stimulant drugs of all types which may be devoid of analgesic activity will also produce a Straub tail reaction in mice. This is really a suggestive technique for the detection of analgesic activity. If the response is present, the material may or may not be analgesic.

4. A study of action current in nerves will give an index of suppression of sensation at the periphery. Pure sensory nerves must be used for this mode of evaluation.

5. A test measuring uricosuric effect, using the output of uric acid as an index of analgesic activity, could aid in the selection of compounds of the aspirin type. Some substances which produce a uricosuric effect are extremely weak analgesics, if analgetic at all; therefore, this does not constitute a specific analgesic technique.

6. Recently, a technique has been developed in our laboratories by Randall and Selitto (20) for the detection of analgesic activity. This method is one which is sensitive both to potent analgesics and to the weak analgesics. The technique is a combination of a mechanical method for evaluating analgesia and a physiological response method. It combines the administration of yeast (as a phlogistic) in the plantar surface of one paw of a rat, and the use of a pressure device for eliciting a response. The threshold in the foot that has been injected with yeast suspension drops to less than one-half that of the control foot one hour after the injection of yeast.

The apparatus, illustrated in Fig. 6, consists of aneroid gauge connected through a T tube to a 10 ml. syringe. To the plunger of the syringe is affixed a bullet-shaped wooden peg. To the inlet of the system there is attached a needle valve. With this valve fully open, the air pressure is adjusted by means of a diaphragm control so that the pressure increases within the system at a rate of 20 mm. of mercury a second. This rate of pressure increase is critical. The pressure in the system at response may be read off the aneroid gauge.

This system has been tried in other laboratories and, because they have permitted the rate of pressure increase to vary, their results have not been in accord with those originally reported by Randall and Selitto (20). Once they have adjusted their rate to the prescribed rate of increase, the data have been corroborative.

Usually the right hind paw is used for the administration of yeast suspension. The left hind paw is used for reference. The following observations are made on the animal: The temperature of the hind feet, the circumference of the inflamed foot and normal foot, and the threshold for response to pressure of both feet in mm. Hg. Usually, the animal shows pain by attempting to escape. The response will

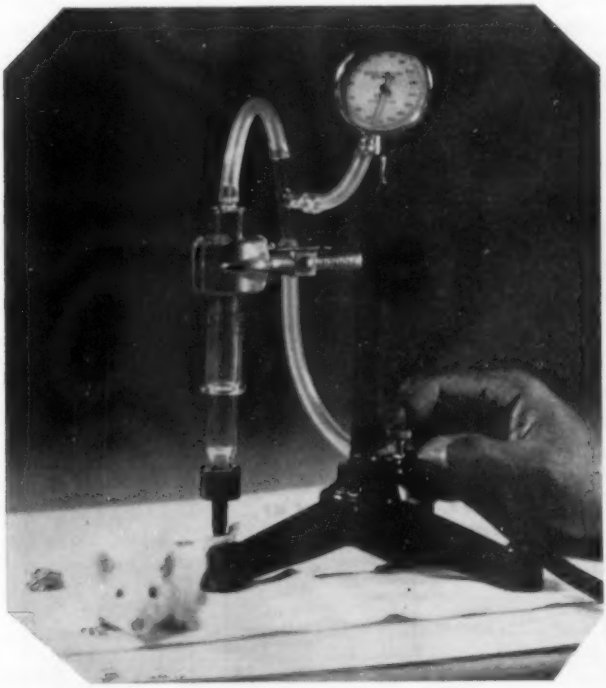

FIG. 6.

vary from animal to animal but the escape reaction is obvious. The temperature of the skin of the back is also taken in order to determine whether or not the general skin temperature of the animal has changed. The results obtained by using this technique with Dexedrine ® administered at 25, 12, and 6 mg./kg. is presented in Fig. 7 for the inflamed foot and the "normal" foot in millimeters of mercury expressed as change from pretreatment value, so that from 0 to 210 mm. Hg. expresses the increase in the threshold with the Dexedrine ® for the inflamed foot; whereas, from 0 to 130 mm. Hg., the increase for the normal foot. This increase in both feet indicates a central effect. Anti-inflammatory activity is also present when Dexedrine ® is given at 25 mg./kg. since there is 60 per cent decrease in the anticipated edematous response.

Temperature changes are indicated in the lower part of the graph. The temperature of the back is shown (lower right) indicating that skin temperature has dropped slightly in these animals over

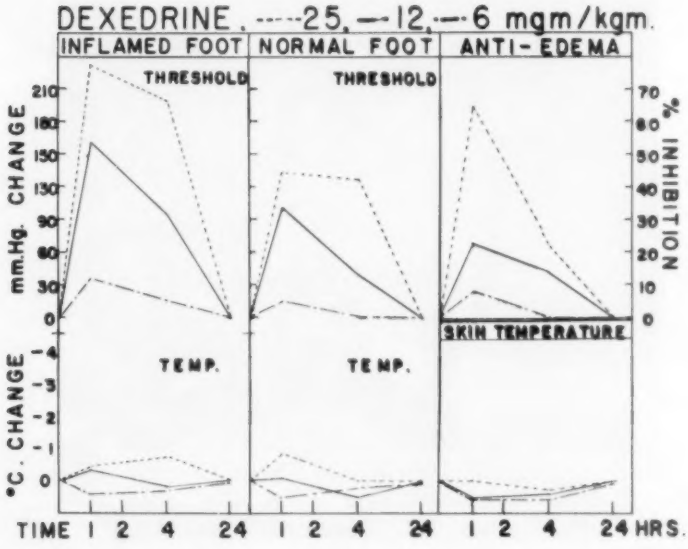

FIG 7.

a period of four hours. Temperature of the normal foot and of the inflamed foot showed inconsistent variation. Dexedrine ® appears to have what can be interpreted as analgesic activity in this test. This probably is related to the effect of the drug on the cerebral cortex. It may be similar to the "analgesia" that has been described under stress conditions attributed to the outpouring of epinephrine.

When phenylbutazone is studied, Fig. 8, we note that in the inflamed foot there is an increase in pain threshold, no change in the normal foot; a definite decrease in temperature of the inflamed foot; a slight but inconsequential increase in temperature in the normal foot, very little change in the skin temperature of the back, and a decrease in the edema produced by the administration of yeast. In this case, anti-inflammatory activity is demonstrated.

Fig. 9 shows the results obtained with alphaprodine, or Nisentil ®, showing an increase in reaction threshold in the normal foot, and an increase in the inflamed foot. With phenylbutazone, there was essentially no change in the normal foot. This is inter-

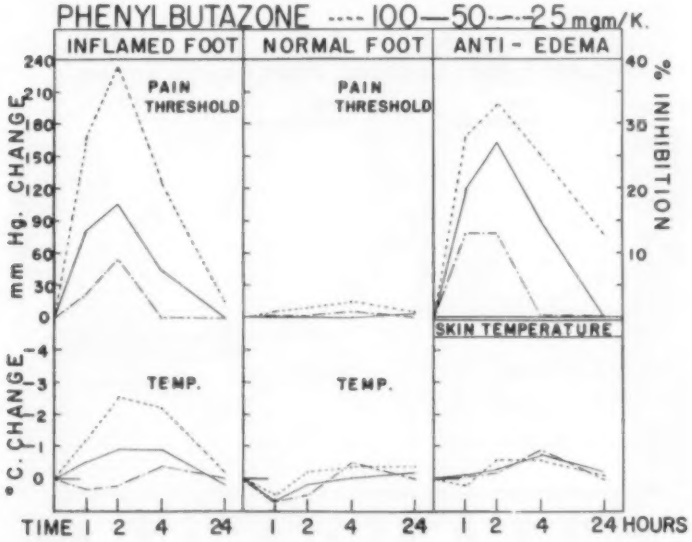

FIG. 8.

preted as distinguishing between the centrally acting materials and the peripherally acting analgesics. With peripheral acting analgesics, there is little or no increase in reaction threshold in the normal foot, and a reduction in reaction threshold in the inflamed foot. The temperature change alters little or none from the "normal" or from the pretreatment control. Anti-edema activity is absent with alphaprodine. Inhibition of response to pain is central since an increase of threshold occurs in both the inflamed foot and the normal foot, and no change in either the degree of edema or foot temperature.

Fig. 10 provides a comparison of sodium salicylate and sodium salicylate plus caffeine, showing the changes that can be discerned with this technique in potentiation of analgesia when combined with caffeine. The ED_{50} would, of course, be less for the combination than it is for sodium salicylate if potentiation is present. This graphic presentation demonstrates potentiation of analgesia, antipyretic and anti-inflammatory action.

Fig. 11 shows the broad spectrum of analgesic agents which can be detected with this technique ranging from the stronger centrally

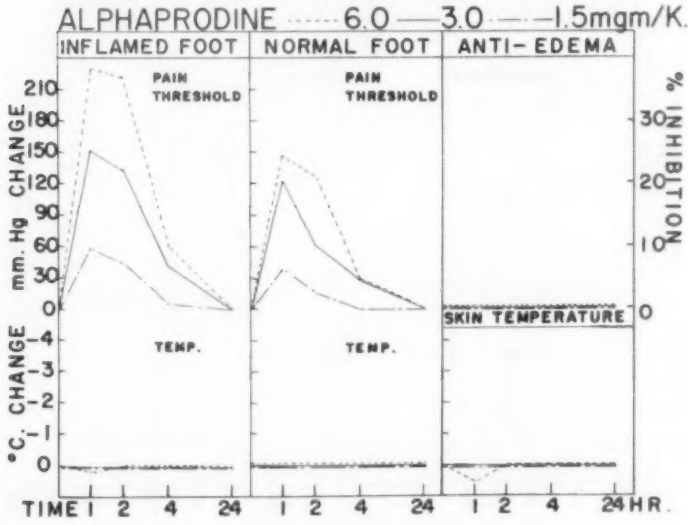

FIG. 9.

acting analgesic substances down through those with weaker action. The latter are considered as peripherally acting analgesic substances as indicated by this technique. Sodium salicylate and aminopyrine are of the same order of potency.

d-Propoxyphene (not indicated in Fig. 11) in this test demonstrates an ED_{50} at 50 mg./kg., so that there may be a deficiency in the test in that chemical compounds of d-propoxyphene type are not accurately evaluated in view of the claim made that d-propoxyphene is of the order of activity of codeine as well as the suggestion of greater potency in the phenylquinone writhing test.

Fig. 12 presents a comparison of sodium salicylate, phenylbutazone, and aminopyrine showing the dose response curves that can be obtained with this technique. Ro 2-5383 (1,3,6-Trimethyl-8,8-diphenyl-1,2,3,4,5,6,7,8-octahydropyrido[4,3-d]pyrimidine oxalate) is a phenyl pyrido pyrimidine derivative.

Randall and Selitto feel that they have introduced a technique which for the first time gives the pharmacologist the opportunity to do dose response studies on the weak analgesic substances.

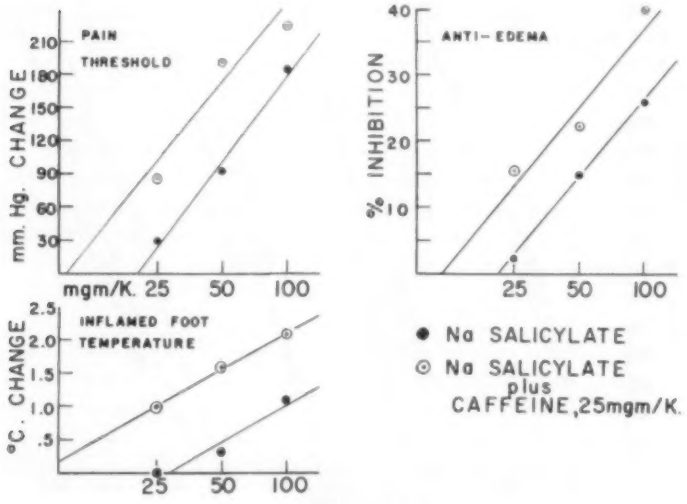

FIG. 10.

In conclusion, I would like to state that there are good techniques for the evaluation of compounds having potent analgesic activity. The Randall-Selitto technique holds promise as a method for selecting the weaker type of analgesic substance. We would like to have a technique for evaluating the intermediate potency compounds and for evaluating compounds capable of obtunding Pfeiffer's classification "sympain". Methods presently available do not appear to select these specifically so that, if we are working in the field of the moderately active analgesic of the d-propoxyphene type, we may have difficulty. In our laboratories at present we are using a combination of the Randall-Selitto test and the Hardy group test for the evaluation of chemical compounds. In other words, if we get the type of response with the Hardy group technique that we get with codeine, and similar potency to codeine and d-propoxyphene in the Randall-Selitto test, we would perform additional evaluation studies and, if most tests were indicative of analgesic activity, we would submit them to clinical trial. The difficulty is that these compounds lie in a potency range for which we are not sure we have an adequate testing method.

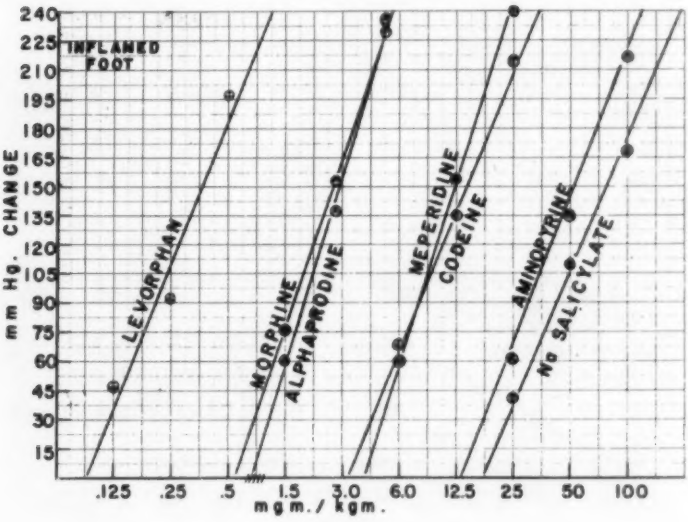

FIG. 11.

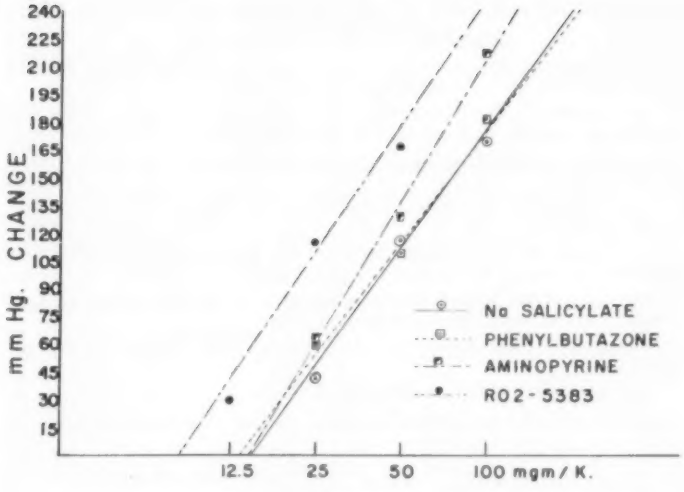

FIG. 12.*

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Acknowledgment

I would like to thank Dr. L. O. Randall for permission to use the data obtained by the group under his direction presented in the portions of this discussion on phenylquinone writhing and the "inflamed foot" technique.

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NEW NITROGEN MUSTARDS IN THE MANAGEMENT OF HODGKIN'S DISEASE

By John R. Sampey *

NITROGEN mustards account for more than 60 per cent of all cases of Hodgkin's disease under chemotherapeutic management. Methyl-bis-(beta-chloroethyl)-amine, or HN2, and methyl-bis-(beta-chloronaphthyl)-amine, or R48, are two of the older N-mustards employed most frequently. The present study presents results on the control of Hodgkin's disease with the newer N-mustards. Several of these will be shown to induce better and longer remissions, and to be less toxic to the patient.

Chlorambucil

Chlorambucil, or CB1348, (N,N-di(2-chloroethyl-p-aminophenyl-butyric acid) is the most widely used of the new nitrogen mustards in the management of Hodgkin's disease. Bernard and associates (6, 7) have released two publications on the treatment of 52 patients with this mustard: they record 17 prolonged remissions, 15 moderate improvements, and 20 showing no effect. Marchel (7) in discussing this report states that he found no response in one patient on CB1348 therapy. Doan *et al.* (20) in narrating their experiences tabulate 22 good remissions, nine fair, with one patient out of 47 not being able to tolerate the drug. Miller *et al.* (44) noted 11 marked objective responses and eight moderate, while 20 of 38 patients showed complete subjective responses and eight, some improvement; the average duration of the objective responses was just short of 12 weeks; nine patients received a second course of CB1348, and six responded significantly; of 11 patients receiving a combination of adrenal steroids and chlorambucil, four had significant responses.

Israels *et al.* (33) observed only four good remissions in 35 patients with Hodgkin's disease after CB1348 therapy, but 21 showed some benefit. Rottino (50) reported 14 regressions of tumors in 31 patients, with five others showing subjective improvement. Bouroncle

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et al. have published two studies on the action of the drug. In one (8), they stated that patients with Hodgkin's disease responded better than those with acute and chronic leukemia, multiple myeloma, and other blood diseases; there were 40 patients in this group, but no data were given on the number responding. In a second report (9), they described excellent responses in six of 24 patients with Hodgkin's disease, and marked improvement in nine. Galton *et al.* (25) noted that 23 patients tolerated the aromatic N-mustard well, but that only four showed striking remissions. DeVries (18) in 1956 recorded that three patients in seven with Hodgkin's disease had good remissions and three, fair; in 1958, he (19) treated 13 patients and observed eight good, three slight, and one moderate response. Hall *et al.* (30) concluded that 16 patients with Hodgkin's disease found chlorambucil less effective than did those suffering from lymphomas and chronic lymphocytic leukemia. Aguirre and Silva Sosa (2) claimed eight complete remissions and two partial in 11 patients with Hodgkin's disease. Haddow (29) described preliminary tests with the drug on nine patients, and reported no significant results; in a second series of six patients with Hodgkin's disease and four with lymphomas, all showed some response except one.

Ultmann and associates reported few good remissions in three studies of CB1348 therapy: in one study (62), they observed one good and five slight responses in six patients with Hodgkin's disease; in a second release (61), they found only one partial remission in six cases, and in a third study (63) only two fair improvements in eight cases. Consoli and Violante (14) observed three good and one partial response in eight patients. Lovisetto and Castellano (43) caused four good but brief remissions in seven patients. Olsen (47) noted five brief responses in seven cases, but all showed marked leukopenia and anemia. Desai (15) classed his results with CB1348 as three complete and two partial remissions in six cases, and Fusco (24) evaluated his results as four good and two fair remissions in six cases. Three brief responses were tabulated by Libansky *et al* (41) in five cases, and Pillers and Magalini (49) noted some fall in lymphocytes and platelets in seven patients with Hodgkin's disease. Gumport *et al.* (28) observed marked benefit in two patients and some improvement in a third following CB1348 therapy of three cases. Desaive *et al.* (16) had two out of three patients show transient improvement after intra-arterial injections of chlorambucil. Desmonts

(17) concluded the drug was not as effective as older N-mustards in two patients with Hodgkin's disease. Klvana (36) induced a six-months' clinical remission in one of two patients with a combination of x-rays and CB1348. Brief improvement has been recorded in four patients by two reports of Hansen (31, 32).

Nitrogen Mustard Oxide

Nitrogen mustard oxide, or nitromin, is the second most used drug among the new N-mustards in the treatment of Hodgkin's disease. Lovisetto *et al.* (42) have described two good and six partial remissions in 10 patients. Eckhardt *et al.* (22) obtained two objective and two subjective responses in eight patients. Aboul-Nasr (1) evaluated three responses as good, two as moderate, one as poor, and one as no effect in seven patients given nitromin alone while, with a combination of this mustard and cortisone, he noted four good and one fair remission in five patients and, on the combination of nitromin, plus cortisone, plus Thio-TEPA (thio-triethylene phosphoramide), he described one moderate response in one patient. With a combination of x-rays and nitromin, Schwenkenbecher (53) obtained four improvements to nine months in six patients. Sellei and Eckhardt (56) recorded one subjective and objective response in five patients, using a combination of nitromin and degranol. Consoli published three investigations on the management of Hodgkin's disease with nitrogen mustard oxide: in 1954, he (12) recorded good remissions in most of four cases of Hodgkin's disease and five of leukemias and, the same year, he (11) noted three good remissions in three cases of Hodgkin's disease; in 1955, he (13) observed some good improvement in four other cases. Klvana (36) induced clinical remissions in three of five patients for one, eight, and ten months. Koyama and Toknyama (37) described objective improvement in one of four patients treated with nitromin. Stoll (59) found one partial response in two cases. Dudik (21) and Mrazek *et al.* (45) each reported fair improvement in single cases of Hodgkin's disease. Other reports on the use of nitromin in Hodgkin's disease include that of Nasr and Awad (46) who observed that two of their three patients were restored to normal activity. Apel and Klages (3) treated 33 malignant diseases with the drug, including one Hodgkin's disease, but they did not report the number responding.

Degranol, B. C. M.

Degranol, or B.C.M., (1,6-bis(betachloroethylamino)-1,6-deoxy-D-mannitol dihydrochloride), has induced some remarkable remissions in patients with Hodgkin's disease. Eckhardt *et al.* (23) described 22 complete remissions to two years in 59 patients. Sellei *et al.* conducted several investigations on degranol: in 1956, they (55) reported six excellent and four fair remissions in 14 patients and, in 1958, they (57) recorded 51 complete remissions for an average duration of three months, with some extending to two years in 59 patients. Benko (5) noted four objective and two subjective responses in 15 patients. Szentklaray (60) employed a combination of degranol and vitamin C to induce nine good and one fair objective response, and 10 good and one fair subjective improvement in 14 patients on B.C.M. Zulik (65) evaluated his 12 responses in 15 patients as good. Sandor *et al.* (52) observed partial remissions lasting as long as 16 months in eight of 11 patients, and they stated that the new mustard was less toxic than TEM. Barlow *et al.* (4) noted eight good and one fair response in 10 patients on degranol.

N-Mustard Phosphoramide Esters

Brichta *et al.* (10) administered the nitrogen mustard phosphoramide ester, B518, to secure two remissions, lasting two and 12 months, in seven patients with Hodgkin's disease. Gross and Lambers released two clinical studies on the action of phosphoramide esters: in one (26), they found four partial responses in four patients, using N,N-bis(betachloroethyl)-N′,O-propylendiamid ester of phosphoric acid, and, in the other, they (27) reported that three of four patients showed some improvement. Schwenkenbecher (54) observed a good remission in a patient on the N-mustard phosphoramide, Endoxan, which lasted two years.

Embichin and Novoembichin

Lareonov *et al.* have released several studies on the action of embichin (2-chloroethyl-methylamine) and novoembichin on Hodgkin's disease: in 1956, he (38) treated 60 patients and reported 53 were alive after one year, 33 after three years, and eight after five years; in 1958, he and Ziv (39) stated that three-fourths of 18

patients in the early stages of the disease lived three years, nine lived five years, and two lived eight years. Also, 22 patients in the middle stages and 10 in the advanced stages showed the following results: one-third survived over three years, and two survived over five years. In 1959, Lareonov (40) treated 50 patients with these two mustards and he noted that 25 survived three years, 11 lived four years, five lived seven years, and two more than eight years; he concluded that, if chemotherapy is begun early, the survivals are longer than when x-rays are administered first.

Uracil Mustard

Shanbron *et al.* (58) tested the uracil mustard, U8344 (5-bis-(2′-chloroethyl)amino-uracil) in the management of 23 patients with Hodgkin's disease: they recorded good but brief responses, and they concluded the drug was not as effective as in chronic leukemia.

N-Mustards Related to Chloroquine and Quinacrine

Jones *et al.* (34, 35) published two releases on the management of Hodgkin's disease with N-mustard derivatives of chloroquine and quinacrine, two antimalarial drugs; they found some improvement in one of two patients on the former, but judged the drug was too toxic; quinacrine was without effect on one patient.

N-Mustards CB3025 and CB1506

Papac *et al.* (48) conducted clinical trials on two new N-mustards: with CB1506 (di-2-chloroethyl methanesulfonate), they observed slight improvement in two of three patients and, with CB3025 (di-2-chloroethylamino-L-phenylalanine), they noted a transient regression of lesions when the drug was given intravenously to one patient and, orally, it induced partial remissions in seven of 13 patients.

Benzimidazole Mustard

Ultmann *et al.* (64) reported that the action of 2(di(2-chloroethyl)-aminomethyl)benzimidazole was ineffective and toxic in one patient with Hodgkin's disease.

Summary

Table I summarizes the results in the management of 927 cases of Hodgkin's disease with the newer N-mustards. In evaluating "good" and "fair" remissions, three factors have been weighed: namely, (1) improvement in the clinical condition; (2) effect of the drug on the blood picture; (3) duration of the response. Almost one-half of the 65 published reports have dealt with the action of chlorambucil. Nitromin therapy has the highest remission rate, while degranol has the highest proportion of good remissions. Three of the most used of the new N-mustards have a higher remission rate than that noted for all N-mustards in the management of Hodgkin's disease (51).

TABLE I

CONTROL OF 927 CASES OF HODGKIN'S DISEASE WITH NEW N-MUSTARDS

N-mustard	*Total No. of Cases*	*No. Not Evaluated*	*Good Rems.*	*Fair Rems.*	*Rem. Rate*	*No. of Refs.*
Chlorambucil	444	56	109	137	62%	30
Degranol	197	—	108	20	65%	8
Embichin	160	—	—	102	64%	3
Nitromin	66	—	21	26	73%	15
Uracil	23	23	—	—	—	1
Phosphoramide ester	16	—	1	9	—	4
CB3025	14	—	—	8	—	1
CB1506	3	—	—	2	—	1
Benzimidazole	1	—	—	—	—	1

Acknowledgments

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SELECTED ABSTRACTS

Diamino-Diphenyl Sulfone as an Antimalarial. Archibald, H. M., and Ross, C. M., *J. Trop. Med. Hyg. 63:*25 (1960). Diamino-diphenyl sulfone has been found to have a beneficial effect upon malaria as well as its established dramatic effect upon leprosy. The effect upon malaria was first suspected when it was found that leprous patients receiving diamino-diphenyl sulfone exhibited an unusual feeling of well-being. Investigation of malarial parasite densities in leprous patients under treatment with diamino-diphenyl sulfone, as compared with non-lepers in the same villages, showed that the lepers had no *P. malaria* trophozoites in the peripheral blood and that *P. falciparum* trophozoites had been reduced to about $\frac{1}{10}$th the density as compared with the non-lepers.

This finding prompted a further study in school children in whom a single dose of 200 mg. of diamino-diphenyl sulfone was compared with 300 mg. of chloroquine base, given as the phosphate. Although chloroquine showed more rapid antimalarial activity, diamino-diphenyl sulfone was able to achieve trophozoite clearance from the peripheral blood. The authors also pointed out that larger doses might be more effective. Doses of 800 mg. weekly have been given for years in the treatment of leprosy without significant ill effects. The drug is also low in cost.

The authors postulated that the drug probably acts against the malarial parasites by competing with PABA for certain essential enzymes in the parasite.

Oral Activity of Steroid Increased by Oily Vehicle. Alibrandi, A., *et al., Endocrinol. 66:*13 (1960). Several steroids, including testosterone esters, androstanolone and certain of its esters, 17 α-methyltestosterone, prednisone, and prednisolone and certain of its esters, were administered to rats once daily by stomach tube in the form of either an oily solution or an aqueous suspension. The androgenic compounds were evaluated on the basis of their effects

on the seminal vesicle, ventral prostate, and levator ani weights in castrated male rats. The cortisone-related compounds were evaluated on the basis of their effects on the thymus, spleen, and adrenal weights of intact female rats.

The authors found that nearly all of the androgenic compounds and some of the cortical compounds showed a greater degree of specific activity when administered orally in an oily solution than when administered as an aqueous suspension. It was also found that the effectiveness of the compounds was increased by esterification with short-chain fatty acids. The amount of the lipid solvent did not influence the activity of any of the compounds.

Although the authors found no direct evidence to explain the increased activity of the compounds tested when given in oily solution as compared with aqueous suspensions, they suggested some possible explanations. It may be that steroids are absorbed more slowly when in oily solution and, therefore, are present in the blood stream for a longer period of time than when given in aqueous suspension. Esterification probably also reduces the rate of absorption. Another possibility is that the oil vehicle protects the steroids from transformations during the digestion and absorption processes.

BOOK REVIEWS

Oxidation—Reduction Potentials of Organic Systems. W. Mansfield Clark. xi + 584 pp. The Williams and Wilkins Co., Baltimore, Md., 1960. Price: $13.50.

Dr. Clark was quite accurate in stating that this book is intended to be both readable and a text. The first few chapters are concerned with a brief history, definitions, and background information relating to the study of oxidation-reduction potentials. The following chapters discuss oxidation-reduction phenomena associated with the formation of dimers and intermediate free radicals (and the necessary mathematical manipulations required), coordination compounds and liquid junction effects. The standard hydrogen half cell completes the theoretical treatment.

One chapter is devoted to techniques, including potentiometry, various half cells and electrodes in popular use, purification techniques and the use of indicators. The chapter dealing with criteria of reliability of redox potentials is best summarized in the author's own words, "more experiments are needed."

Following a chapter on miscellaneous, uncategorized topics is the unreadable or "texty" portion of the book—about 250 pages of tabular data, culled from the literature, representing potential studies of various organic systems.

The appendix consists of a few pages of various constants, conversion factors, thermodynamic data, mathematical relations and a glossary of symbols. An extensive bibliography and index complete the text.

Some readers may question the somewhat scanty treatment of polarography as a technique for the study of organic systems, considering the wealth of data available on this topic. However, many texts specifically devoted to this area of study are readily available.

A familiarity with the concepts of thermodynamics is, of course, necessary for a full appreciation of the material presented in this book.

A. R. GENNARO

Principles of Human Pathology. Edward B. Smith, Parker R. Beamer, Frank Vellios, and Dale M. Schulz. xi + 1123 pp. Oxford University Press, New York, N. Y. Price: $15.00.

In a dynamic new approach to the study of pathology, this text integrates the facts of the basic sciences with the clinical features of diseases. Besides the basic pathologic information presented, the authors have attempted to interpret the student's knowledge of biochemistry, physiology, and anatomy with disease processes.

The sixty-nine chapters within this text could be divided into two general categories: The first twenty-nine chapters can be designated as a consideration of general pathologic states, including a chapter on the history and meaning of pathology. An understanding of this section is a prime requisite for the comprehension of the remaining chapters which deal with the disease states of specific organs and/or systems of the human body.

Each chapter is well written and easily understood, and each chapter is further enhanced by the presence of adequate and ample references at its termination.

While primarily intended as a textbook in human pathology, this book will also serve as a valuable reference source for all scientists engaged in medical research.

E. E. Vogin

Principles of Biochemistry. Abraham White, Philip Handler, Emil L. Smith, and DeWitt Stetten, Jr. xiii + 1149 pp. The McGraw-Hill Book Company, Inc., New York, N. Y., 1959. Price: $15.00.

As scientific knowledge of the human body continues to expand, it becomes evident that every manifestation of the biological activity of living cells results from underlying chemical processes. Biochemistry, a relatively recent science, has enabled researchers to understand and to appreciate more fully the chemistry of living matter and, especially, the human body. It is, therefore, the intention of the authors that this book give emphasis to the study of the principles of biochemistry as a basis for the understanding of, and application to, medicine.

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Of particular note is the manner in which chemical concepts are presented in conjunction with a biological or biochemical principle, and the fact that the necessary principles of physical chemistry are included within the framework of the text, rather than in a separate introductory section.

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